TAXONOMY AND BIOLOGY OF PHLEBOTOMINE VECTORS OF HUMAN DISEASE

ANNUAL REPORT

DAMD17-82-C-2223

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DECEMBER 30, 1985

Supported by

U. S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND

Fort Detrick, Frederick MD. 21701-5012

UNIVERSITY OF FLORIDA
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Taxonomy and Biology of Phlebotomine Vectors of Human Disease		5. TYPE OF REPORT & PERIOD COVERED Annual 30 Dec. 1984- 30 Dec. 1985
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) David G. Young		8. CONTRACT OR GRANT NUMBER(s) DAMD17-82-C-2223
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Florida Gainesville, FL 32611		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Ft. Detrick, Frederick MD 21707-5012		12. REPORT DATE 30 Dec. 1985
		13. NUMBER OF PAGES 88
14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Sand fly   Lutzomyia
Phlebotominae
Leishmaniasis

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

In the Americas, phlebotomines from the forest canopy of Peru, various sites in Colombia, Venezuela and Brazil were studied. In Peru, the undescribed females of two sand flies were discovered and a male, belonging in the medically important flaviscutellata complex, apparently represents a new species. Undescribed taxa from the other countries were also identified and several taxonomic papers were begun or completed during this period. New distributional records of phlebotomines based on these collections and from colleagues in Brazil were added

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to the information already compiled for the handbook of the American Phlebotominae.

Using established methods, two species of sand flies, L. shannoni and L. intermedia (a suspected leishmaniasis vector) were easily reared from wild-caught Brazilian females. Two other suspected vectors from Colombia continue to be maintained in the laboratory. A female of one of these species, L. spinicrassa, was found naturally infected with Leishmania b. braziliensis in eastern Colombia. Parasites from the gut, inoculated into a hamster, were later identified by isozyme profile as this subspecies of Leishmania. This discovery is significant because previously L. wellcomei, occurring only in Brazil, was the only sand fly which had been found naturally-infected with this important Leishmania. Additional isolates of a new arbovirus from sand flies from eastern Colombia were made in 1985. Flight range and longevity of sand flies from this site (Arboledae) were studied during this time and a manuscript on the subject was completed.

An up-to-date review of phlebotomines as vectors of leishmaniasis in the Americas was submitted for publication (draft enclosed in present report). Three other papers dealing with experimental infections of Leishmania in sand flies from the U.S.A. were completed.

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Introduction

This report covers the period from 30 Dec. 1934 to 30 Dec. 1985. Research was focused mainly on New World sand flies with the aim of completing a handbook on the neotropical fauna.

Objectives

1. To study the taxonomy of sand flies from the Neotropical Region, with special emphasis on the fauna of Mexico and Central America, and to provide a handbook on the entire fauna.

2. To study the taxonomy of sand flies from Africa and the Near East, providing identification keys, reference collections (based manily on newly collected material); and to evaluate future needs for study in these areas.

3. To colonize, in the laboratory, different species of Phlebotominae from Africa, the Near East and the Neotropical Region, with special emphasis on vector species.

4. To assess the susceptibility of colonized species to aetiologic agents of disease.

Results

The following results are discussed in order of the objectives given above.

1. Taxonomic studies of Phlebotominae.

a. New World

The PI continued to receive phlebotomines collected by Dr. Terry Erwin (USNM) in the forest canopy of eastern Peru. This study is especially interesting because of our limited knowledge on the vertical distribution of New World sand flies and because some of the reservoirs of leishmaniasis are arboreal. So far, 28 Lutzomyia and 1 Brumptomyia species have been recovered from these canopy fogging collections (Oct. 1982-present) but there is no evidence that any of them, except possibly Lu. moucheti, is a true canopy dweller. We have recovered the previously unknown females of this species and Lu. omagua from these collections as well as two undescribed taxa in the genus, one of which (1♂) belongs in the medically important Lu. flaviscutellata complex. This new information will be included in the handbook of neotropical Phlebotominae (in preparation).

Surprisingly, two male sand flies were captured taking blood meals from an entomologist sitting in the canopy elsewhere in Peru (March, 1985; Junin Prov., Mission Cutivereni). This observation represents the first such indication that males can puncture the skin of a vertebrate for the purpose of obtaining blood. Further studies are needed to evaluate the importance of this observation, perhaps significant in view of earlier findings of unidentified organisms, probably Bartonella, on the mouthparts of male sand flies in an endemic area of bartonellosis in Peru (Hertig, 1940. Am. J. Trop. Med. 22: 1-81. The two males from Junin Province belong in the vexator species group and are closely related to, but distinct from, Lu. peruensis -- a suspected vector of bartonellosis in Peru.

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A review of the Peruvian sand flies, written by the PI and two Peruvian colleagues, was published in 1985 (copies enclosed). Continuing studies of Peruvian sand flies will be enhanced by collaboration with a U.S. Navy Research Unit in Lima and Iquitos and with Enrique Pérez of the Alexander Von Humboldt Tropical Medicine Institute who will be studying with the PI for two months in 1986.

Field studies, including collections of sand flies for taxonomy, in Bahia State, Brazil, in late October and early September, 1985, yielded 19 Lutzomyia species. The previously unknown females of Lu. pelloni and Lu. viannamartinsi were collected and an apparent new species in the subgenus Psychodopygus was examined (♀ only). The field sites lie within a highly endemic area of cutaneous leishmaniasis, presently being studied by investigators from WRAIR and the University of Brasilia. The PI will enlarge the identification key to the sand flies of this area based on these and other new findings. Plans were made to compare isoenzyme profiles of Lu. shannoni from Bahia, Colombia and Florida.

Dr. Jorge Arias of INPA, Manaus, Brazil, kindly provided the PI with many unpublished records of phlebotomines in the Amazon basin. The distribution maps for the identification handbook were accordingly modified to reflect this new information on about 75 species.

Dr. Dora Feliciangeli de Pinero from Venezuela studied with the PI during May, 1985. She amassed a large collection of sand flies from the poorly-collected Amazon region of southern Venezuela. At least three new species were examined (Fig. 1-3) and will be described in two forthcoming papers. Valuable specimens from Venezuela were added to the collection at the University of Florida.
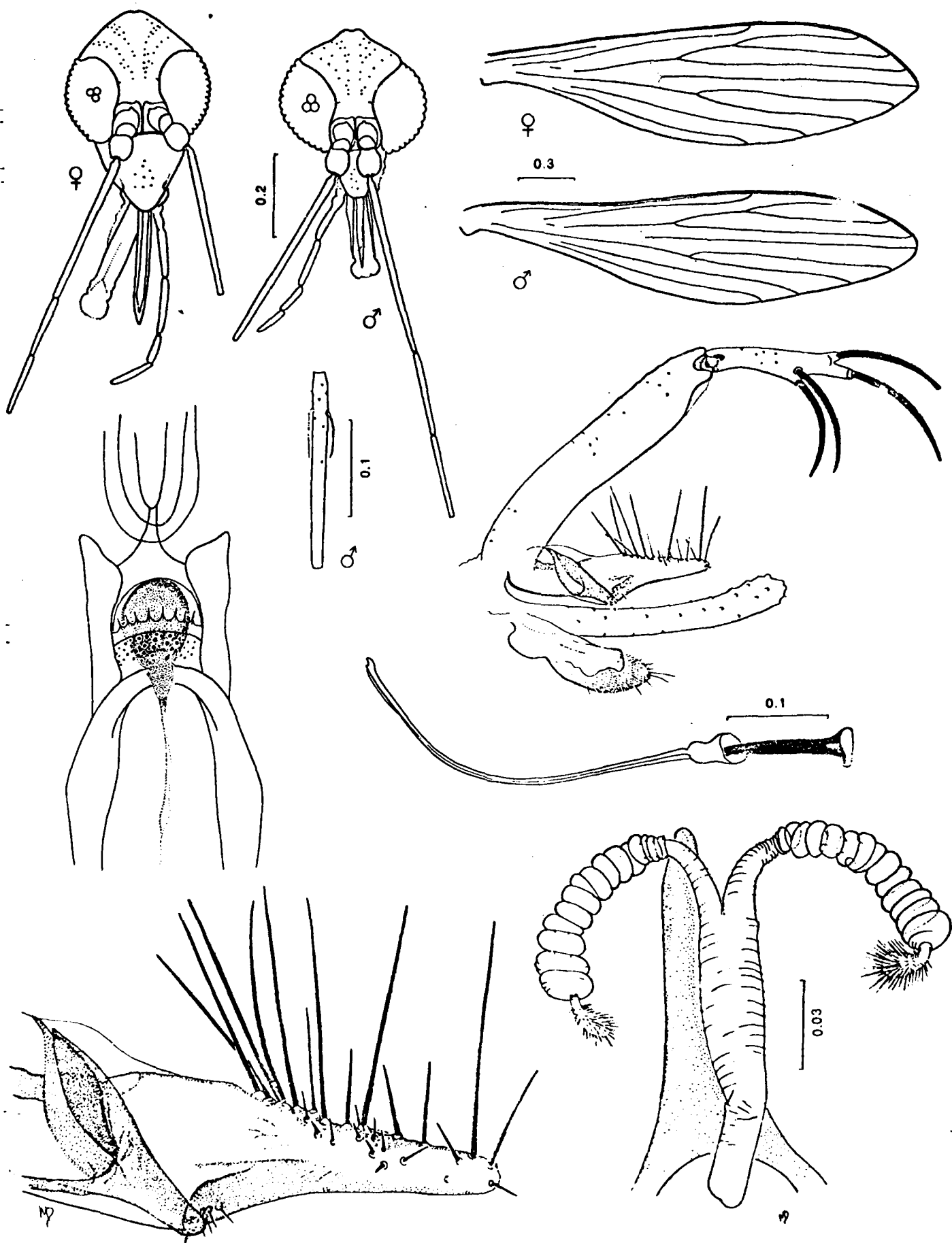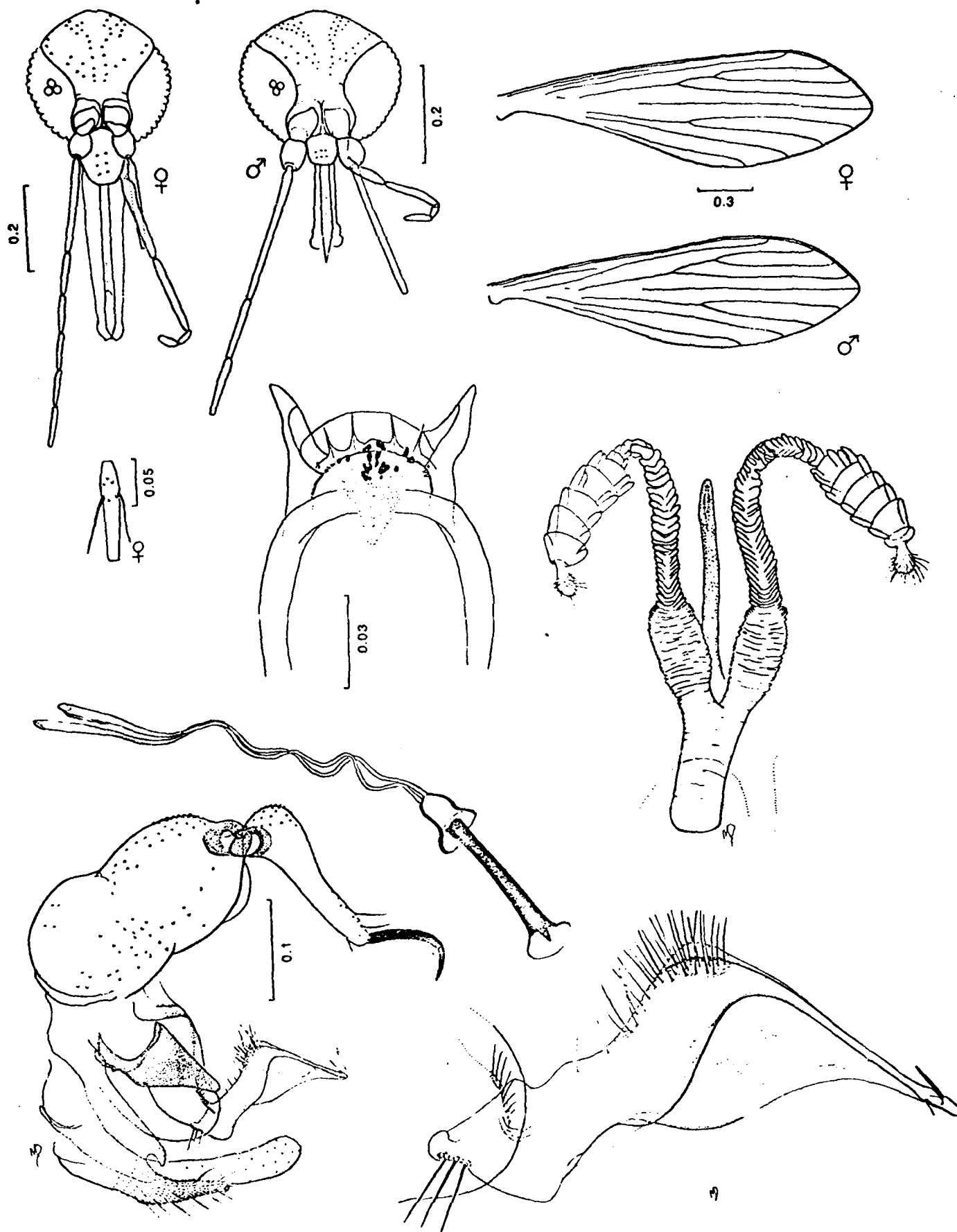
FIG. 1. Lutzomyia (NYSSOMYIA) N.SP.
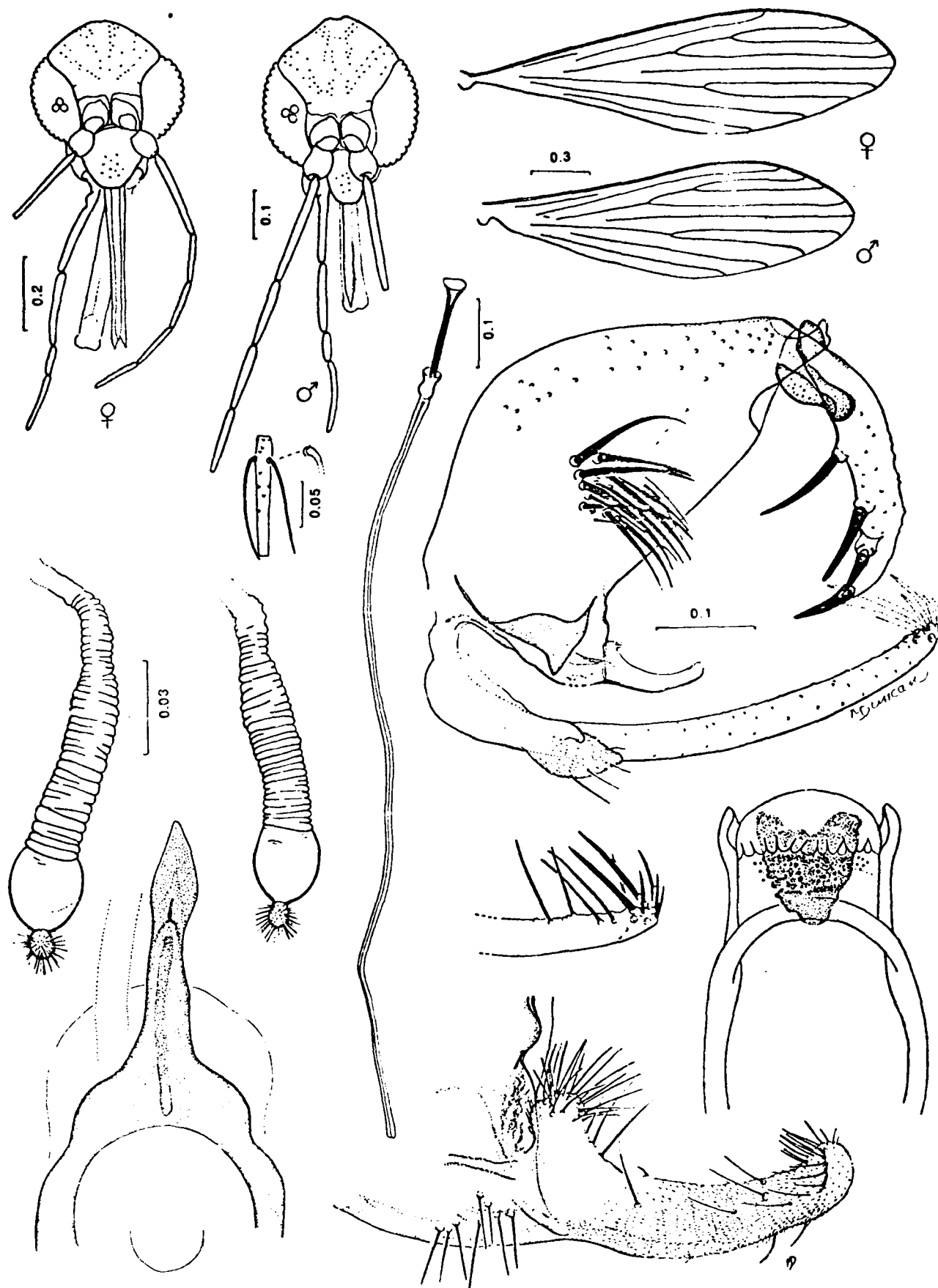
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Fig. 2. LUTZOMYIA (PSYCHODOPYGUS) N.SP.

FIG. 3. LUTZOMY (TRICHOPHOROMYIA) N. SP.

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In Colombia, the PI collected sand flies in Feb., July and Oct., 1985. A good series of an undescribed sand fly in the subgenus Trichopygomyia was obtained in eastern Colombia (Fig.4,5). Six other species were found for the first time in the country. The females of Lu. cerquerai and Lu. erwindonaldoi were previously unknown; a paper describing them and the new species is being prepared. Illustrations were completed.

b. Old World

The PI did not collect phlebotomines in the Old World during this period nor were any specimens sent for identification. In view of the recent publications reviewing all the Old World Phlebotomus species (Lewis, 1982, and Artemiev & Neronov, 1984; received 1985) no effort was made to study these insects. The Artemiev & Neronov book, written in Russian, will be translated in 1986 according to an agreement with a local translator.

2. Colonization of sand flies

In October near Gandu, Brazil, eggs were obtained from resting sand flies. Most were kept in Brasilia under the care of WRAIR investigator. A few (<100) were brought to the University of Florida where they hatched. Larvae grew well on a standard diet (Young et al., 1981. J. Med. Ent. 18:446). Adults were subsequently identified as Lu. intermedia, a suspected vector of cutaneous leishmaniasis in southern Brazil, and Lu. shannoni. None of the adults laid viable eggs but only three F_1 females of Lu. intermedia took blood meals.

Productive lab colonies of Lu. gomezi and Lu. spinicrassa from specimens originally collected by the PI and colleagues in Columbia, continue to be maintained at YARU, Yale University where colonies of
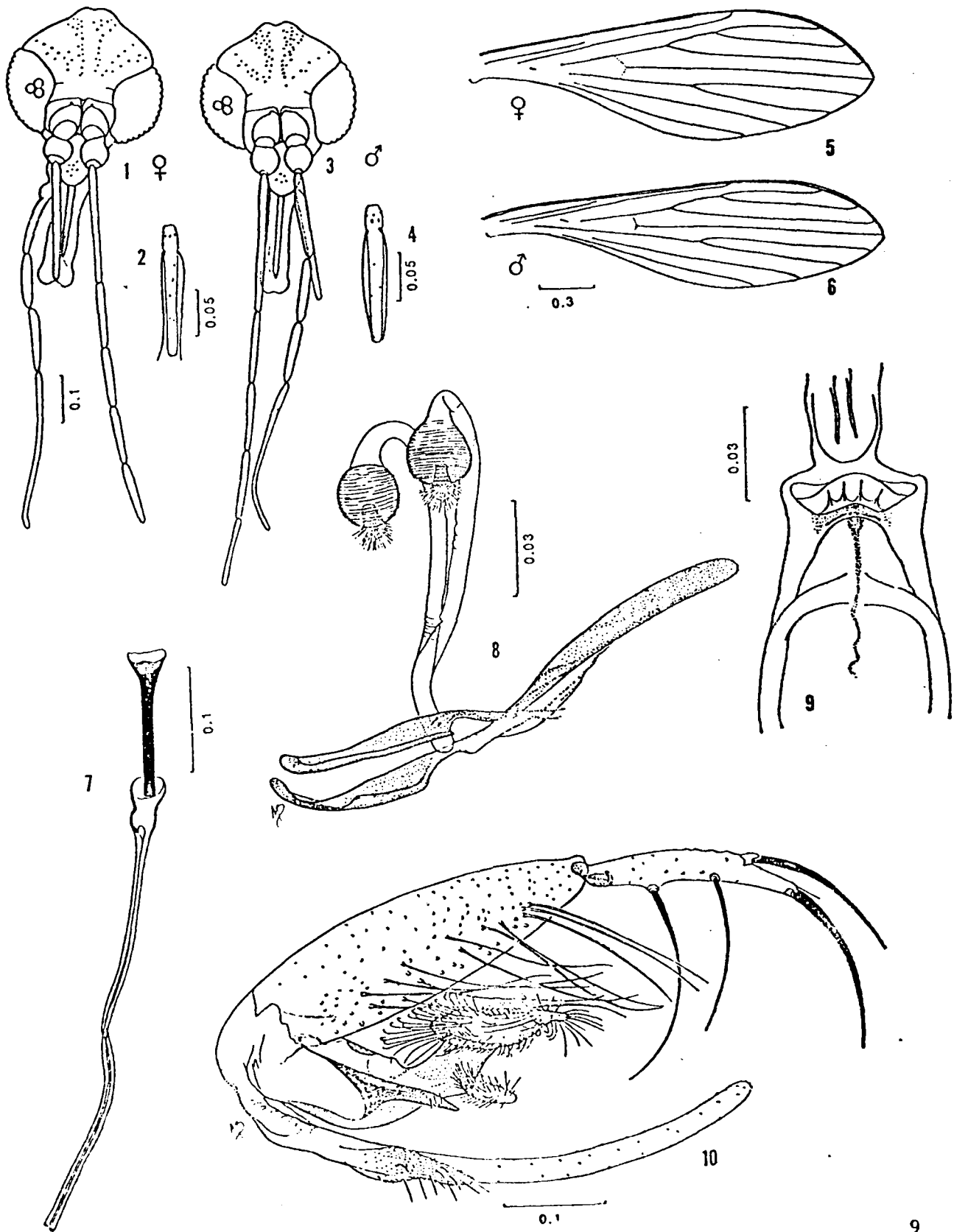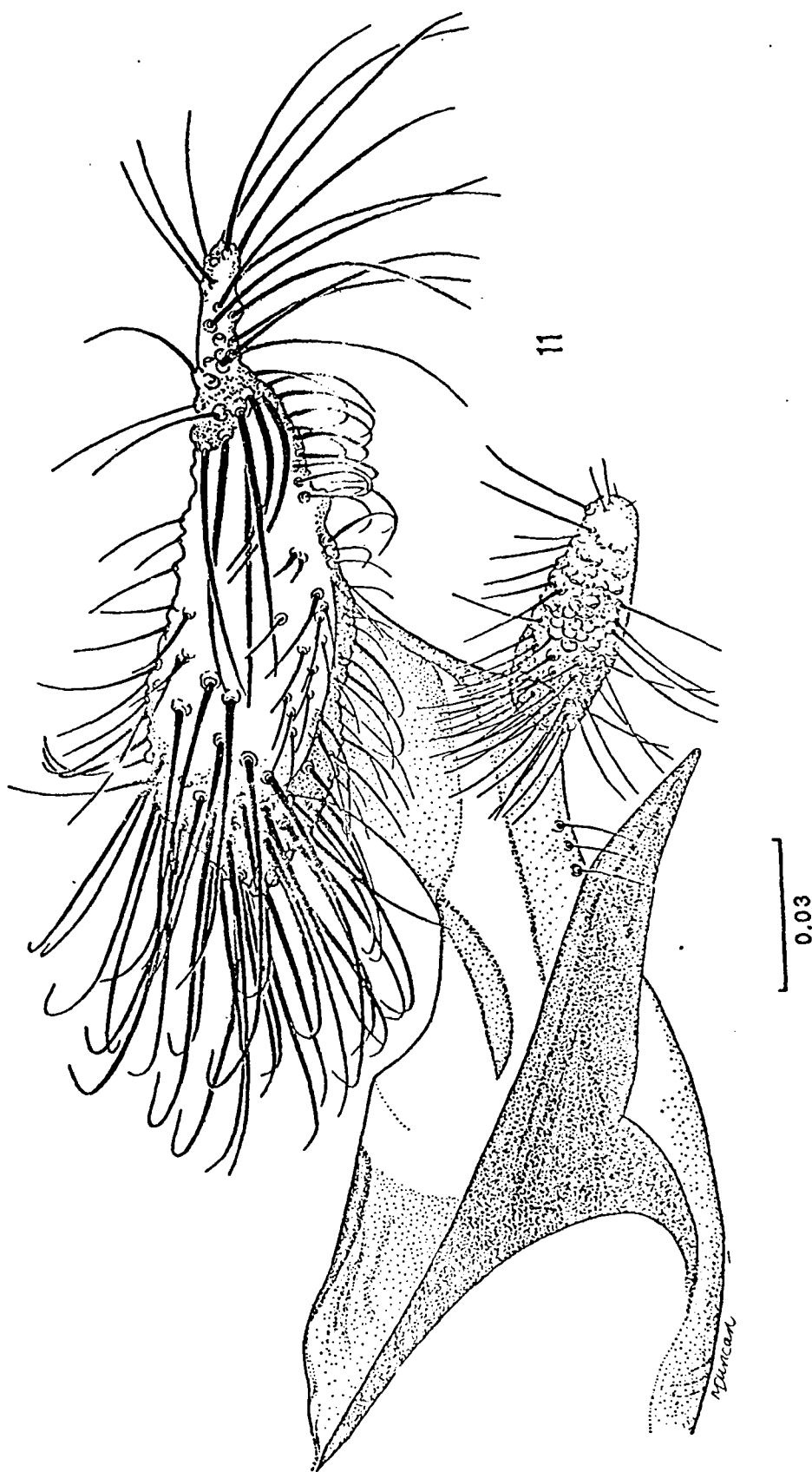
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FIG. 4. LUTZOMYIA (TRICHOPYGOMYIA) N.SP.

FIG. 5. PARAMERE OF LUTZOMYIA
(TRICHOPYGOMYIA) N. SP. ♂

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such exotic species are permitted. *Lutzomyia vexator* is presently
being reared at the University of Florida.

3. Disease Relationships

An up-to-date review of phlebotomines as vectors of leishmaniasis
in the Americas was completed (Appendix I) and will be published as a
chapter in "Current Topics in Pathogen-Vector-Host Research" in 1986.
Another article (with Drs. R.G. Endris and P.V. Perkins) on the
experimental transmission of *Leishmania mexicana* by a North American
sand fly, *Lutzomyia anthophora* was also submitted for publication (Am.
J. Trop. Med. Hyg.) in 1985. Two other articles on the development of
Leishmania in sand flies under laboratory conditions were completed.

Epidemiological studies on cutaneous leishmaniasis caused by
Leishmania b. braziliensis continued in Norte de Santander, Colombia.
Previously, the only sand fly incriminated as a proved vector of this
important, widely-ranging parasite was *Lu. wellcomei* -- a sand fly with
a limited geographic distribution in Brazil (some localities in the
States of Pará, Amazonas and Ceara). Obviously other sand fly species
must be involved in transmission outside the range of *Lu. wellcomei*.

At Arboledas, Colombia, where *Leishmania b. braziliensis* is
endemic, *Lu. spinicrassa* of the *verrucarum* group is the most common
anthropophilic species. Since 1 Jan. 1985, we have dissected more than
3,000 females of *Lu. spinicrassa* and an equal number of 14 other
species. These were all collected at endemic foci near Arboledas,
cryopreserved in liquid N_2, and eventually dissected at the PI's
laboratory in Gainesville. One female of *Lu. spinicrassa*, dissected on
28 Jan. 1985, contained paramastigotes attached to the hind gut
epithelium and had several hundred free living promastigotes in the

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mid gut. No parasites were observed in the mouthparts. A hamster, inoculated with these parasites, developed a slight swelling of the nose by Nov., 1985, at which time leishmanial promastigotes were recovered in culture. Subsequent identification of the parasites by isoenzyme analysis (Dr. R.D. Kreutzer) confirmed that the infection represented Le. b. braziliensis. Thus, Lu. spinicrassa is the only sand fly, other than Lu. wellcomei in Brazil, that has positively been found naturally infected with this important Leishmania. A paper giving more information on this discovery will be published in 1986 by the PI and colleagues. Additional isolations of a new Phlebovirus from sand flies collected at Arboledas, Columbia, with the help of the PI were made in 1985.

Field studies on the dispersal and longevity of Lutzomyia sand flies were begun in July, 1985, at Arboledas. Preliminary results indicate that the majority of marked flies (28 out of 55 flies recovered) were recovered within 24 hours of release. Several males of Lu. serrana flew at least 150 mm from the release point within 48 hours of release. A manuscript on this neglected subject was completed in December, 1985 (J.B. Alexander).

APPENDIX I

NEW WORLD VECTORS OF THE LEISHMANIASES

INTRODUCTION

The leishmaniases in the New World are a group of enzootic and zoo-
notic diseases caused by morphologically similar parasites in the genus
Leishmania (Protozoa: Trypanosomatidae). Mammal reservoirs, of which
there are many species (76), may or may not show signs of infection
(24, 52, 71). Furthermore, some of the leishmanial species are host
specific and have not been reported in people (eg., Le. mexicana enriettii
of domestic guinea pigs). Those that do, cause an estimated 400,000 new
cases each year throughout the world (178). Clinical symptoms in man,
including those of cutaneous, mucocutaneous and visceral disease, vary
considerably depending on the species of Leishmania, immunological
responses of the individual and other factors (178). Putative vectors
of these diseases are sand flies in the genera Lutzomyia (New World) and
Phlebotomus (Old World); yet, incrimination of specific vectors and
mammal reservoirs remains undetermined in many foci (66).

One of the vexing problems complicating epidemiological studies,
particularily in the Americas, is that two or more leishmanial taxa and
similar appearing nonleishmanial trypanosomatids coexist in many localities
(73). Moreover, the number of taxa continues to increase as more foci are
studied (72). Identifying these organisms and associating them with
vectors has been difficult, especially before isoenzyme analysis and
other biochemical means of parasite identification became widely used
within the last few years (28, 70, 108, 116, 157, 186). For example,
parasites from only six out of 812 sand flies found with natural

flagellate infections in Panama were conclusively identified as
leishmanial (24).

New foci continue to be discovered, sometimes well beyond the
known geographic range of a particular Leishmania species. The
presence of Le. braziliensis braziliensis, for example, was recently
confirmed for the first time in Central America in Belize (33) where
previously Le. mexicana mexicana was the only parasite known to cause
human disease. It is not always clear whether these new findings
represent long established, but undetected, foci, or else reflect recent
introductions of disease (eg., canine leishmaniasis in Oklahoma, USA).

The existence of sympatric morphospecies or Lutzomyia further compli-
cates epidemiological studies, though seemingly not to the extent as
that shown by the asexual Leishmania. For example, females of L. wellcomei,
a proven vector of Le. b. braziliensis, are difficult to distinguish from
those of L. complexa in Brazil using morphological characters alone (131).
There are additional examples in other species groups and it is likely
that morphospecies or sibling species are more common in the genus than
previously suspected. The few isoenzyme or alloenzyme analyses of
Lutzomyia sand flies, reported so far (123, 131, 164), have shed little
light on this subject nor have any of the results been of epid.miological
significance (131). However, further studies using these methods and those
based on monoclonal antibodies, cuticular hydrocarbons and genetic probes
are expected to provide important information on inter - and intraspecific
variation, especially as it relates to vector competence.

Transmission of leishmaniasis takes place in a variety of habitats
in the New World and results of epidemiological studies in one locality

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may not always apply to another, even within similar habitats in the same country. This is especially true for the Le. mexicana complex and Le. braziliensis complex parasites. Major changes in the landscape, usually resulting from man's activities, obviously affect species diversity and population densities of vectors and reservoirs. Ready et al (132), studying adult sand flies, recently documented the effects of one such change in northern Brazil. With few exceptions (45, 140-143), we know practically nothing about the ecology of the immature stages of vectors, a serious handicap in terms of understanding population dynamics and for developing control strategies.

As a result, our discussion focuses mainly on adult sand flies and their role as vectors or leishmaniasis from the USA to Argentina where autochthonous human cases have been reported (178). We have drawn information from published and unpublished reports, emphasizing those that have appeared in the last decade and which are not readily available. There are several recent reviews on the leishmaniases (72, 73), including historical coverage of the American forms, a topic that we do not fully treat in the present chapter.

CLASSIFICATION OF AMERICAN PHLEBOTOMINAE AND LEISHMANIA

Arguments for treating Phlebotominae as a family of Diptera (1, 2, 136) have been strengthened by a recent study (27) showing that the spermatozoan ultrastructure of three Phlebotomus spp. differs from other psychodids in the subfamily Psychodinae. It will be necessary, however, to study these structures in other subfamilies before conclusions can be drawn. The generic and subgeneric classification of Phlebotominae is even more controversial. Most American sand flies described before the

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mid 1960's were placed in the genus Phlebotomus, but Theodo. in 1965 (163), as he did in 1948 (162), pointed out structural differences between species in this Old World genus and those of Lutzomyia in the New World. We recognize two other American genera in the subfamily, Warileya (6 spp.) and Brumptomyia (24 spp., including 1 undescribed sp. from South America). sand flies in these smaller genera, unlike those in Lutzomyia, have not yet been implicated in disease transmission.

The 392 described and undescribed Lutzomyia spp. known to us are placed into 27 subgenera and equivalent species groups (100, 191). Some of these categories are recognized as genera by specialists who adopt Forattini's classification in whole or part (36, 37). It is difficult, however, to support the position of authors who recognize Psychodopygus, originally created as a subgenus, as a genus without simultaneously raising the rank of equally distinctive subgenera of Lutzomyia, and of Phlebotomus which are just as relevant to this discussion (100). Subgenera and species groups of Lutzomyia containing suspected or proven vectors of the leishmaniases are shown in Table 1.

Although the genus Lutzomyia is large and heterogeneous based on adult structure (163), it remains to be determined if it is polyphyletic. A better knowledge of the morphology of immature stages, including larval polytene chromosomes (177), may help set generic limits but, at present, the larvae of only 34 Lutzomyia spp. have been described (46, 169 & many other references). Similarly, few studies on egg structure and variation have been reported (172, 199).

The infrageneric classification of Leishmania proposed by Lainson & Shaw (74, 75), with later modifications (72, 76), has become widely

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accepted. The species and subspecies are grouped into three taxonomic sections according to where they develop in their vectors. Thus, _Leishmania_ in the Section Hypopylaria (2 spp. infecting Old World lizards) multiply and develop in the hindgut only. Transmission from infected fly to lizard may be by ingestion (64) but this has not been demonstrated. Most _Leishmania_ in the New World are placed either in the Section Peripylaria (_Le_. _braziliensis_ complex) or in Suprapylaria (_Le_. _donovani_, _Le_. _hertgi_ & _Le_. _mexicana_ complexes). The taxonomic position of _Le_. _herreri_, discovered in Costa Rican sloths (195), remains undetermined (72). Peripylarian leishmanias attach to the hindgut cuticle of sand flies but they also move foward to the mid and foreguts, eventually reaching the head region. The suprapylarian parasites, on the other hand, do not attach to the hindgut wall, though slender promastigotes are sometimes observed moving freely in the lumen. Development and multiplication occur initially in the midgut with later dispersal to the head region (see next section). _Leishmania_ _henrici_, discovered in the blood and cloaca of _Anolis_ lizards from Martinique, W.I. in 1912 (87), may not belong in this genus (30) and no other saurian _Leishmania_ occurs with certainty in the Western Hemisphere.

The application of subspecific names for leishmanial taxa, though used in the present chapter following Lainson (72), may not be entirely correct because some subspecies are sympatric (73). It is expected that nomenclatural changes will soon be proposed, not only for this reason, but also because some of the named taxa may be junior synonyms or earlier described forms, notably those in the _Le_. _mexicana_ complex (73, 116). In addition, not all parasites referred to as _Le_. _b_. _braziliensis_ (in the

broad sense) may belong in this taxon and new names may have to be created for them (73). Reference strains of some Leishmania, in effect, serve as neotypes and have added some stability to the classification of these parasites (178).

DEVELOPMENT OF LEISHMANIA IN SAND FLIES

Experimental infections of Leishmania have been studied to varying degrees in more than 30 Lutzomyia spp. collected in nature or reared in the laboratory (17, 24, 61, 64, 76 119, 122, 170). Improved methods for producing large numbers of sand flies in the laboratory (32, 67, 133) have not only allowed investigators to adequately study these host/parasite relationships; they have also provided sufficient specimens for cross-breeding experiments (175), xenodiagnosis (19, 24, 188), age grading and other biological studies (103, 105, 130, 134).

Sand flies are pool feeders, the mouth parts and feeding mechanisms having been earlier described (58, 96). The number of leishmanial amasti-gotes ingested during the blood meal may be critical for the successful establishment of infections within them. This varies according to the species of Leishmania and other factors (64). For example, Franke et al (39), homogenized L. longipalpis females immediately after they had fed on hamsters experimentally-infected with Le. m. amazonensis and Le. b. braziliensis. The homogenates were then streaked onto the surface of blood agar plates and after incubating for one to two weeks the isolated promastigote colonies were counted. Only 22 to 33% ($\bar{X}$ = 16%) of the flies that had fed on Le. b. panamensis ingested amastigotes (from 2 to 16/fly). In contrast, 23 to 100% ($\bar{X}$ = 61%) of flies that had en-gorged on Le. m. amazonensis - infected hamsters ingested leishmaniae

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(from 4 to > 250/fly). The length of the sand fly mouth parts in relation
to the distribution of amastigotes within the host's skin may also have
a bearing on the number of parasites ingested. The fascicle of a mis-
identified Lutzomyia in the verrucarum group (not L. townsendi) ordinarily
penetrates the skin of hamsters to a depth of 130mm which is within the
zone where amastigotes of Le. m. garnhami are especially abundant (17).

Subsequent development of Leishmania in sand flies was recently re-
viewed (64, 115) and only a brief summary on this complex subject will
be given here. Following the infecting blood meal, amastigotes probably
divide one or more times before transforming into promastigotes in the
midgut (64). Those of the Le. braziliensis subspp. invade the pylorus
and, less frequently, the ileum of the hindgut. There, they transform
into parasites (paramastigotes) having expanded, but shortened, flagella.
This is a modification associated with the formation of hemidesmosomes
within the flagellar sheath and serves to anchor the paramastigote to
the hindgut wall, presumably a nutrient-rich site (64). Multiplication
by binary fission continues to occur but, eventually, free living
promastigotes are produced which then move forward to the foregut and
beyond, where, once again, they appear as paramastigotes attached to the
cuticular lining (64). Unlike these peripylarian parasites, those in
the Section Suprapylaria grow mainly in the midgut, becoming attached
to it at times, by inserting their unmodified flagella between microrilli
or occasionally penetrating epithelial cells (115). Although there is
no hindgut development, forward migration follows the same pattern as
that of the peripylarian leishmanias (64). The rate of extrinsic develop-
ment varies with temperature and species of Leishmania. Much work

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remains to be done on the physiology of sand flies in relation to leishmanial susceptibility, as influenced by digestion, peritropic membrane formation, lectins, and endosymbionts (63, 115).

Short active promastigotes, believed to be infective forms (64, 145), may appear in the sand fly's cibarium and mouth parts as early as four days following the infecting blood meal. It is assumed that these forms originate from paramastigotes attached to the pharynx or cibarium (115), appearing when nutrients in the blood meal are depleted. It has been suggested that ingested sugars may block the attachment of paramastigotes at these sites, thus allowing the freely moving promastigotes to more easily invade the proboscis (115). If proven to be true, then the presence of sugars has an obvious effect on transmission. In addition, there is mounting evidence indicating that paramastigotes may interfere with labral and cibarial sensilla (65, 97), thus causing infective flies to probe more often than noninfected flies (9, 65). Further discussion of some of these observations are given by Bray (13).

INCRIMINATION OF VECTORS

Criteria for incriminating vectors of disease have been discussed by many authors including Killick-Kendrick & Ward (66) who applied these guidelines specifically to sand flies and the leishmaniases. Although there is general agreement on the validity of these criteria, the task of gathering evidence is often long and laborious.

Anthropophily

Because transmission of leishmaniasis normally occurs during probing or feeding of an infective sand fly it is obvious that a vector

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must, to some degree, be anthropophilic. This is determined by direct

observations (many reports in the literature) or less frequently by

analysis of sand fly blood meals (23, 24, 76, 160, 161). About 90 of

the 392 Lutzomyia spp. have been reported biting people at one time or

another and, of these, 23 are suspected on proven vectors (Table 1).

More species will certainly be added to this list as additional studies

are completed.

Curiously, leishmaniasis in people or dogs has been reported from

some localities where no mammal feeding sand flies have been discovered

[eg., Guadeloupe, W.I. (26) and Oklahoma, USA (4)]. This may be due to

limited vector search, to imported cases being erroneously considered as

autochthonous, or to other unknown reasons. In foci where only one

anthropophilic sand fly species is present (eg., L. diabolica in Texas,

USA and Northern Mexico; L. christophei in the Dominican Republic), that

species obviously must be considered the likely vector to man, even

before additional incriminating evidence is obtained. However, in most

endemic areas of the New World, two or more anthropophilic sand flies

coexist, yet it is not always the most common one that is responsible for

transmission. Lutzomyia flaviscutellata, an oft-cited example of an

infrequent man-biter, is, nevertheless, the only proven vector of Le.

m. amazonensis to small mammals and man in northern Brazil (73). The

incidence of human disease is understandably highest in foci where the

vectors are strongly anthropophilic (eg., L. verrucarum and perhaps L.

peruensis in the Peruvian Andes). Among other reasons why some anthro-

pophilic species, such as L. davisi in the Amazon basin, have not been

implicated in transmission is that the adults may have a short life span;

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their host range may exclude Leishmania - infected mammals or they may not be innately susceptible to infection.

Natural Leishmanial Infections in Sand Flies

Repeated isolations of Leishmania, indistinguishable from those causing human disease, from anthropophilic sand flies not only provide strong evidence for incriminating vector species (66), but also serve as a basis for estimating the risk of infection at a given time and place (91, 182). Traditionally, female sand flies are individually dissected and examined for promastigotes which are later identified following in vivo or in vitro cultures (178). An overall infection rate is then calculated, but ideally a more realistic and significant value should be based on the proportion of parous females found infected. However, at present no long term investigations of this kind have been carried out in the Americas.

Attempts to culture promastigotes are not always successful even when known leishmaniae, observed in sand flies or mammals, are inoculated into syrian hamsters or suitable culture medium. The infective stage of the parasite may be absent (145) or the parasite itself may not be adaptable to these cultures (72).

Many of the difficulties associated with isolating leishmanias from their intrinsic or extrinsic hosts may soon be overcome by the use of newly developed immunoassays based on highly specific monoclonal antibodies. McMahon-Pratt et al (109) and Frankenburg et al (40) demonstrated that promastigotes from experimentally-infected flies can be rapidly and accurately identified by these techniques. It is not necessary to

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culture Leishmania for these determinations; in fact, it is likely that promastigotes in dry preserved sand flies can be identified as was demonstrated for Plasmodium sporozoites in mosquitoes (15). Furthermore, it should be possible to simultaneously identify sand flies, especially females of sibling species, using these or similar methods.

Meanwhile, cryopreserving field-collected sand flies in liquid nitrogen is an excellent, but little-used, method for holding specimens until they can be dissected in the laboratory, usually under better conditions (112, 118). Promastigotes in sand flies remain viable for months (D. Young, unpublished data), and probably for years, when held at -196°C. Freezing sand flies in the field allows more time for collections and observations, time otherwise spent dissecting, and reduces or eliminates the need for transporting hamsters, culture media, and dissecting equipment to remote field sites. In this regard, light weight cryogenic tanks, lined inside with adsorbent material to prevent the spilling of liquid nitrogen, are particularily useful.

Triturating groups of sand flies to recover Leishmania has been done in some studies (22, 51, 73) and, no doubt, saves time when the only objective is to recover parasites. Yet, there is a danger of mixing two or more species in a single pool; only an estimated infection rate can be calculated; and no information can be obtained on the age structure of the sand fly populations using present methods, i.e. follicular dilatations of individual females (93, 99, 103, 134).

Demonstration of Transmission by Sand Flies and Other Arthropods

Stable flies or dog flies, Stomoxys calcitrans, are able to mechanically transmit Le. mexicana (and probably other Leishmania as well)

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from hamster to hamster under experimental conditions (77), but this mode of transmission of Le. mexicana is assumed to be rare or nonexistant in nature. These insects, however, feed readily on dogs and their interrupted feeding behavior (16) would favor mechanical transmission of some of the Le. donovani complex parasites which are often abundant in the skin of infected dogs (173).

Sherlock (156) studied a focus of Le. d. chagasi in NE Brazil, concluding that the brown dog tick, Rhipicephalus sanguineus, transmitted the parasite to dogs at localities where L. longipalpis, the proven sand fly vector, was absent. However, promastigotes found in ticks were not conclusively identified as Leishmania. Recently McKenzie (107) observed that Leishmania (Le. d. infantum ?) in dogs living in Oklahoma and Kansas, USA could survive in experimentally-infected ticks of the same species for up to 160 days after their infecting blood meals, and for as long as 130 days post molting (nymph to adult). Furthermore she demonstrated that adult ticks, experimentally fed as nymphs on infected dogs about 30 days earlier, transmitted the parasite by bite to another dog (see later pages). Although these results contradict those of another tick transmission trial (137) additional studies are needed to fully assess the role, if any, that ticks may play in the natural transmission of some of Le. donovani complex parasites.

In general, however, sand flies are regarded as the only important vectors of the leishmaniases. It is apparent that the Le. mexicana complex parasites, under experimental conditions at least, can develop in and be transmitted by, many Lutzomyia species, some of which are not natural vectors (195). This lack of host specificity is perhaps analogous

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to Trypanosoma cruzi and triatomine bugs, nearly all of which are
potential vectors but only those bugs having certain habits and
geographic distributions serve as actual vectors of the disease to man
(88). Examples of Lutzomyia females proven to experimentally transmit
Le. mexicana, or closely related parasites, by bite include: L.
anthophora (R. Endris & D. Young, unpublished data); L. christophei
(61); L. cruciata (181); L. diabolica (86); L. flaviscutellata (174); L.
longipalpis (25); L. panamensis or ally (155, 134); L. renei (25); and
L. shannoni (P. Lawyer & D. Young, unpublished data). Only L.
flaviscutellata and L. o. olmeca are proven vectors of any of Le.
mexicana parasites. The other listed sand flies are suspected vectors
(Table 1) or else do not appear to be involved in natural transmission
cycles. Leishmania d. chagasi, also in the Section Suprapylaria, was
experimentally transmitted to hamsters by lab-bred females of L. longipalpis
(80), previously incriminated as the natural vector on epidemiological
grounds (biting habits, general concordance of geographic distribution
with that of the parasite and other data). Recently, promastigotes from
wild caught L. longipalpis in Brazil (83) and Bolivia (90) were con-
clusively identified for the first time as Le. d. chagasi, thus com-
pleting the chain of evidence needed to establish its position as the
principal vector of New World visceral leishmaniasis.

Development of the peripylarian leishmanias (Le. braziliensis
complex parasites) in sand flies has also been studied, as discussed
previously, but there are no reports of successful experimental trans-
missions. Attempts to infect L. flaviscutellata, an unnatural vector of
these parasites, have failed (73), and probably other, but unreported,

attempts with different Lutzomyia spp. have been equally discouraging. This may be due, in part, to the difficulty in colonizing peripylarian vectors in the laboratory (73), or more likely to the fact that it is difficult to infect high proportions of sand flies with some of these parasites under experimental conditions (39).

Documentation of naturally-infected, identified sand flies transmitting leishmanaisis to man adds further weight to the incrimination of vector species but such reports represent only a few, isolated observations. Pifano et al (125) incriminated L. panamensis as a vector in Venezuela when a collector contracted leishmaniasis (Leishmania sp. undetermined), four weeks after being bitten by 17 wild caught L. panamensis females. Biagi et al (10) reported that a field-collected female of L. o. olmeca (as Phlebotomus flaviscutellatus) transmitted Le. m. mexicana to a human volunteer in Mexico. Additional evidence from there and Belize (182) supported the view that L. o. olmeca is, indeed, an important natural vector in these areas.

Other Epidemiological Evidence

Lutzomyia spp. closely related to proven vectors and with similar host preferences, are sometimes presumed to be vectors in the absence of other evidence. Lutzomyia o. bicolor, for example, has not been found naturally infected with Le. m. aristedesi, but females of this subspecies probably transmit this parasite to rodents, especially Orozomys, at Sasardi, Panama where these animals are commonly infected (55). Like its close relatives in the flaviscutellata complex, L. o. bicolor is readily attracted to rodents (22).

This subspecies and many other sand flies are not known to transmit

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Leishmania to man yet some of them may help maintain infections among
reservoir hosts. However, these enzootic associations and the extent to
which they occur, has been poorly studied in the Americas. Christensen
et al (24) showed that L. shannoni in Panama feeds frequently on sloths
and suggested that this sand fly may serve as a vector of Le. b.
panamensis among these animals, but not to man.

Other observations providing clues to the incrimination of vector
species obviously include knowing where and when transmission takes
place and which age groups of people are most infected. Knowledge of
the habits of people in relation to those of man-biting flies can
provide important information in this regard. Ward et al (176) first
reported that L. wellcomei was an avid day biter at Serra das Carajos,
Para' State, Brazil where the Leishmania infection rate was high among
men working in forests during the day. Subsequent isolations of Le. b.
braziliensis from L. wellcomei, along with other information (73),
incriminated this species as the principal vector.

GEOGRAPHIC DISTRIBUTION OF VECTORS

In contrast to the Phlebotomus spp. in the Old World that live
mostly in temperate zones, the Lutzomyia sand flies are essentially
tropical insects (94). The few species occurring in North America were
probably derived from such stock (192). The present center of distri-
bution of the Lutzomyia spp. lies in the lowland forests of South America,
east of the Andes where 40 or more species may coexist in a single
locality (193). One explanation for this rich diversity is that recur-
ring dry periods during the Pleistocene Epoch, and probably before,
served to isolate conspecific populations of sand flies in wet refugia

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(190). In time, some of these populations became reproductively isolated. Alternating wet periods allowed sand flies to expand their ranges; some of which apparently spread around, not across, the Andes Mountains to colonize areas mostly to the West and North.

Oceanic islands, including those of the West Indies (excluding Trinidad which is a continental island) have depauperate sand fly faunas (35). Most species feed on lizards but a few such as L. christophei, a suspected vector of leishmaniasis in the Dominican Republic and L. orestes, a closely related species known to bite people in Cuba (110) are mammalophilic. Other sand flies, now extinct, once inhabited Hispaniola. Examination of 14 undescribed fossil sand flies of two species (D. Young & R. Johnson, unpublished data) preserved in Dominican amber and believed to be about 26,000,000 years old (146), showed that they closely resemble modern day forms (see Fig. 1 & ref. 127). None of the fossil females has yet been examined for leishmanial infections[

On continental land masses in the neotropics, the sand fly fauna is much richer. However, no sand flies have been reported from Chile (105) and only two species are known from Uruguay (106). Imported human cases of leishmaniases were recently reported in the former country (147).

Of the proven vectors of leishmaniases, L. longipalpis has the widest geographic range (disjunct in dry areas from southern Mexico to northern Argentina) but there is some evidence indicating that it represents a species complex, consisting of two or more taxa (175). The same may be true for L. shannoni which occurs from Maryland, USA to northern Argentina but there are no data to support this hypothesis.
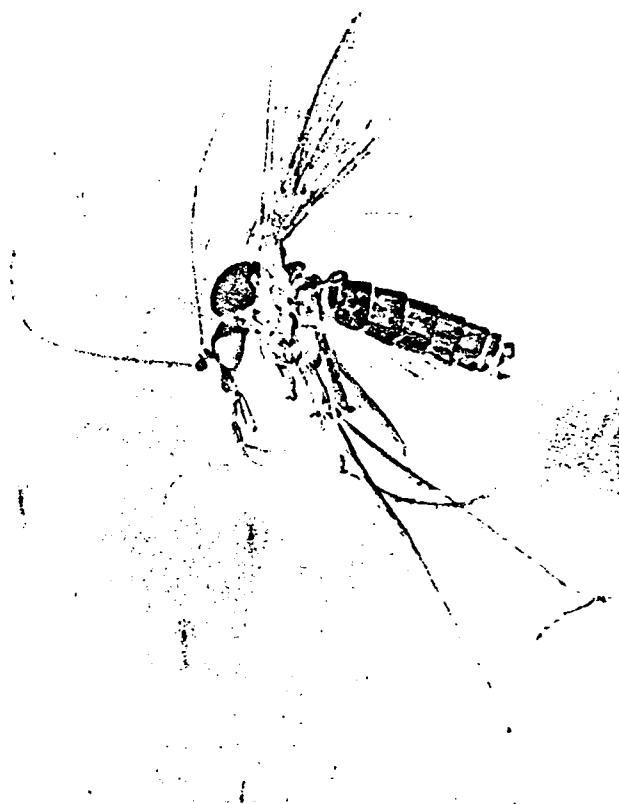
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Fig. 1. An unnamed <u>Lutzomyia</u> female preserved in amber from the Dominican Republic and believed to be approximately 26,000,000 years old.

Some other species, less widespread, have markedly disjunct distributions. An undescribed Lutzomyia sp., previously misidentified as L. townsendi in western Venezuela (D. Feliciangeli; J. Murillo & D. Young, unpublished data) and a suspected vector there (153), was recently discovered in Costa Rica in a coffee growing area where Le. b. panamensis occurs (R. Zeledoy, personal communication). This sand fly has not been reported elsewhere. Further information on the geographic distributions of individual vectors is given later in this chapter.

SOME HABITS OF SAND FLIES AS RELATED TO LEISHMANIASIS

Lewis (94) reviewed this broad subject; here we discuss some aspects of feeding, longevity and flight range, while later giving more information on habits of specific vectors. Killick-Kendrick (63) discussed some aspects of the biology or phlebotomines that have not been well studied.

Although it has not been established where or when adult sand flies obtain sugar meals in nature, it is assumed that both sexes require carbohydrates for energy and longevity (98). Obviously, both factors have an important bearing on the transmission of leishmaniases. The presence of fructose, glucose and sucrose has been detected in the crops of Old and New World phlebotomines (64, 98).

Williams (182) examined 2466 individual sand flies of eight species in Belize, noting that the crops of 155 contained varying amounts of a clear fluid, probably sugar solutions as was determined previously (98). J. Alexander (personal communication) failed to find pollen grains on the heads or bodies of 2371 Panamanian sand flies examined and, therefore, concluded that floral nector does not serve as a usual sugar source for

these insects. Other investigators have drawn the same conclusion and have suggested that extrafloral nectaries, honeydew, ripe fruits and other plant juices may serve as natural sugar sources.

Alexander futher analyzed 554 Panamanian sand flies using the cold anthrone test (166), determining that fructose, at least, was present in 129 males and females of six Lutzomyia spp. The high proportion (58%) of 65 males collected from tree trunks and having such sugar may reflect the possibility that they, like males of mosquitoes, require more sugar meals than females because they do not accumulate fat as adults (167). However, up to 37% of nongravid females from tree trunk collections had ingested detectable levels of sugar.

Ingested sugars are stored either in the flexible crop or else pass directly to the midgut or the fly depending on the depth of penetration of the mouthparts and interaction of the palpus with the surface of the sugar source (130, 148). Some observations (182) indicate that the presence of ingested sugar may help stimulate host seeking of female flies.

In addition to sugars, most Lutzomyia females require one or more blood meals to initiate ovarian development. There is a range of geno-trophic patterns, however, that varies within and among sand fly popu-lations. Johnson (59) studied an obligatory autogeneous subpopulation of L. gomezi in Panama, noting that females did not require a blood meal for the maturation of the first batch of eggs. Lewis (93) and Perkins (122) observed the same phenomenon in a closely related species, L. cruciata, in Belize and Florida, USA, respectively.

Facultative autogeny has been observed in a proportion of L.

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beltrani in Belize (183) and L. shannoni females from Florida (122).

Nearly all (96%) of 349 nonbloodfed L. shannoni reared in the laboratory

deposited up to 40 eggs within four days of emergence. Yet, other

females, containing developing eggs from the same lab colony readily took

multiple blood meals prior to the first oviposition. Such gonotrophic

disconcordance, also observed in anautogeneous species (14), obviously

enhances vector potential, yet the extent to which multiple feedings

occurs during a single ovarian cycle in nature is unknown. The typical

gonotrophic pattern, anautogeny, is exhibited by most Lutzomyia vectors

which require one or more blood meals for the developemnt of all their

eggs.

Host seeking behavior is complex but little studied in sand flies.

The various, but not necessarily separate, events leading to the ingestion

of vertebrate blood include activation, orientation, landing and feeding

(62). Most anautogeneous females are physiologically ready to feed with-

in one to four days after emerging from the pupal stage (31, 67). Suit-

able combinations of light intensity, ambient temperature, relative

humidity, air movement and other physical conditions stimulate hungry

sand flies to search for blood meals.

This behavior varies according to phlebotomine species, vertebrate

host and habitat (126). For nest-inhabiting species such as L. anthophora

in Mexico and Texas, USA, the search may be short because their hosts

occupy the same microhabitat and blood feeding corresponds to the time

when the host is present and probably resting (189). Females of L.

anthophora are unable to fly after a full blood meal and there seems

to be no selection pressure for them to do otherwise.

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In contrast, most other *Lutzomyia* spp. must travel greater distances from resting sites to find their hosts. Forest species such as *L. trapidoi* and *L. umbratilis* search for arboreal mammals by moving in short, hopping flights along tree trunks and branches (21). In the same habitat, other vectors (eg., those in the *L. flaviscutellata* complex) restrict their hunting mostly to sites at or near ground level. *Lutzomyia longipalpis*, a robust sand fly, commonly occurs in treeless localities and is not closely associated with mammal nests. Its flight range has not been determined but it is likely that both sexes fly considerable distances to locate their hosts. This species and others, such as *L. gomezi*, *L. diabolica*, *L. intermedia*, *L. verrucarum*, *L. spinicrassa*, *L. panamensis*, will readily enter houses to bite the occupants (many scattered references). Yet, in general, these and other vectors are exophilic; there are not truly domestic sand flies in the Americas.

In Panama, Christensen et al (21) found that a high proportion of gravid *Lutzomyia* females were attracted to caged bait animals, other than man. They suggested that blood meals taken shortly before oviposition could provide an energy source that would lessen the deleterious effects associated with egg laying. However, there was no evidence that these females actually took blood meals (due to the design of the baited traps), and thus the possibility remains that some of them, at least, may have been attracted to conspecific males resting near the animals. Results of other studies in the neotropical forests indicate that the proportion of gravid females coming to human bait is less than 10% (D. Young, unpublished data).

Orientation to a host probably involves several stimuli including

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host odors (130), temperature gradients (149) and perhaps, pheromones released from feeding females or conspecific males, that for some species, are also attracted to vertebrates (111). Schlein et al (149) demonstrated that Phlebotomus papatasi females secrete an aggregation pheromone, probably from palpal glands, while taking a blood meal. The response to this volatile pheromone, not yet reported in American sand flies, varied according to the temperature of the experimental feeding chamber.

Males of L. longipalpis normally outnumber females on or near their vertebrate hosts (120, 196) and their vigorous wing beating behavior may promote better dispersal of sexual pheromones, believed to be secreted from dorsal abdominal glands (85). Such glands have not been reported in other sand flies but, nevertheless, rapid wing beating seems to be a common premating trait among all male sand flies (11).

The size and structure of sand fly mouth parts can provide clues regarding host preferences. Lewis (96) observed that mammal feeders generally have a long labrum and that it and the paired maxillae possess hooked teeth, rather than ridge-tipped maxillae which are associated with reptile feeders. Most American Phlebotominae are mammal feeders and, with few exceptions, are regarded as opportunistic, feeding on a variety of mammals depending largely on their availability (21, 160, 161). Precipitin testing of sand fly blood meals has provided important epidemiological information on vector host preferences in Brazil (23) and Panama (160, 161) but has been little used elsewhere in the neotropics. Another technique for studying this subject involves inoculating individual vertebrates with rubidium, a relatively stable, nontoxic element that was detected in mosquitoes six days after they had fed on experi-

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mentally-marked birds (69). If this method proves applicable to phlebo-
tomines and mammals, it may supplement precipitin testing of blood meals
and additionally provide data on the dispersal and longevity of sand
flies after they have fed.

Longevity and Flight Range

We knew little about longevity of sand fly vectors in nature and
it is difficult to study this subject there or in the laboratory where
it is impossible to duplicate natural conditions. Nevertheless, studies
of lab-bred phlebotomines have provided some understanding of longevity.

In terms of their own survival, and therefore that of associated
leishmanias, sand flies have evolved mechanisms for coping with periods
of dryness or cold temperatures that, otherwise, would reduce or perhaps
eliminate their populations. These unfavorable periods are not always
predictable, even in the neotropics, and thus no single strategy would
enable all individuals to survive (96). It has been observed tha·
females of some Lutzomyia spp. occurring in both the Neotropical and
Nearctic Regions, lay normal developing and quiescent or diapausing eggs
in the same batch (60, 86). This increases the probability that some
of the progeny, at least, will eclose and live under favorable conditions.
This adaptation has also been observed in other insects (168), the
type of egg produced being dependent on the temperature, photoperiod
(and relative humidity levels?) experienced by the parent during the
preoviposition period (168). Lawyer (86) studied the effects of day
length on adult L. diabolica from Texas, USA in relation to the proportion
of diapausing eggs laid by females (see p.). Although the influence
of photoperiod may be less significant in the neotropics, Johnson &

Hertig (60) observed uneven hatching rates within single egg batches of L. gomezi, L panamensis and L. geniculata when kept under identical conditions. It is also assumed that larvae of some neotropical species respond to unfavorable conditions by becoming dormant but this may not represent true diapause.

From numerous laboratory studies (eg., 60), most adult females of Lutzomia generally live from one to four weeks depending on species, temperature and other factors. There is virtually no available information on the adult life span of sand flies in nature. Chaniotis et al (18) recovered five marked males of the Panamanian species, 15 days after they had been dusted with fluorescent powder; but, their ages were obviously unknown before they were marked. It has been assumed that high female mortality associated with oviposition of lab-bred flies does not occur in nature (64); however, this was not supported by one age grading study of field-collected Phlebotomus papatasi (103).

Similarly, the flight ranges of sand flies are poorly known. Chaniotis et al (18) marked about 20,000 sand flies in a Panamanian tropical forest. Subsequent recapture of marked flies showed that most (ca. 90%) dispersed within 57m of the release point. The maximum distance travelled by a single f , in one day was 200m, the farthest distance monitored from the release point.

LUTZOMIA VECTORS AND ASSOCIATED LEISHMANIA

Leishmania herreri, a parasite of sloths in Costa Rica (195), is not considered further because of its uncertain taxonomic status (72). Lutzomyia trapidoi, L. ylephiletor and L. shannoni are implicated as vectors (195).

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Section Peripylaria (Leishmania Braziliensis Complex)

Most of the peripylarian leishmanias cause single or multiple ulcerated skin lesions in people; mucocutaneous leishmaniasis, due to Le. b. braziliensis, is a serious disease that may evolve months or years after the primary lesion(s), found elsewhere on the body, have healed (178).

LEISHMANIA BRAZILIENSIS BRAZILIENSIS   Lainson (73) noted that human isolates, referred to as Le. b. braziliensis, from the Amazonian lowlands may actually consist of several distinct leishmanias. The reference strain (not a topotype) of this parasite was isolated from a patient at Serra dos Carajas, Para, Brazil, for the most part a relatively high, well drained locality (133). There, Lainson et al (81) found promastigotes in females of L. paraensis (2 positive of 175 examined), L. amazonensis (1 of 127) and L. wellcomei (3 of 1656); however, only those recovered from the latter species were conclusively identified as Le. b. braziliensis. Ward et al. (176), as noted earlier, observed that L. wellcomei females avidly bit man during the day and night. Other specimens were captured in rodent-baited Disney traps but not on tree trunks or in animal burrows. The diurnal resting sites and host preference range of this species remain unknown.

Significantly, Wilkes et al (180) showed that a high proportion (51%) of females collected during the day at this locality were parous and were thus potentially infective. Night collections yielded a lower proportion of parous flies of only 25%. Their findings supported laboratory observations showing that L. wellcomei females ordinarily lay their eggs at night after which they seek another blood meal within 24 hours (180).

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Autogeny in L. wellcomei has not been observed and the females are gonotrophically concordant.

Populations of L. wellcomei, the only proven vector of Le. b. braziliensis so far known, decrease during the dry season at Serra dos Carajas (July - September), a time corresponding to decreased human infection (133). This species also occurs in the Brazilian states of Ceara (135) and Amazonas, near the Rio Urubu (J. Arias & D. Young , unpublished data). However, Le b. braziliensis occurs outside the geographic range of L. wellcomei and thus other vectors must be involved with transmission. Among these suspected vectors (76), L. intermedia, L. pessoai, L. whitmani, and others have been implicated in transmission in parts of southern Brazil (37, 3, 72, 129) L. panamensis in Venezuela (3, 125) and an undescribed sand fly closely related to L. carrerai carrerai in Bolivia (H. Bermudez & F. LePont, personal communication). The latter species also occurs in foci of mucocutaneous leishmaniasis in Peru (as dark form of L. c. carrerai, 193) and in parts of Amazonia Brazil (J. Arias, personal communication). Further characterization of Leishmania isolated from these are required before any of them can be regarded as proven vectors of Le. b. braziliensis.

Recently, this parasite was isolated and identified biochemically (R. Kreutzer, personal communication) from patients living near or in coffee groves at Arboledas, Norte de Santander, Colombia where L. spinicrassa (verrucarum group) and L. gomezi (subgenus Lutzomyia) accounted for 90% of all sand flies collected in man biting collections from April, 1984 to February, 1985 (D. Young, A. Morales & colleagues, uunpublished data). Of 4024 L. spinicrassa females dissected, four contained, as yet unidentified,

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promastigotes. One positive L. gomezi has also been found; promastigotes in it and in two of the L. spinicrassa females were attached to the pylorus and were also seen in the mid and foreguts. This suggests that they may represent Le. b. braziliensis rather than Endotrypanum parasites (154) of sloths because these animals are absent at this locality.

Vector information on Le. b. braziliensis in Belize (33), Ecuador (139) and Paraguay (171) is virtually nonexistant.

LEISHMANIA BRAZILIENSIS GUYANENSIS   The epidemiology of leishmaniasis caused by Le. b. guyanensis is relatively well understood in Northern Brazil (5, 82, 84, 132) and French Guiana (41, 89, 91, 92, 121). The putative vector is L. umbratilis, a forest dwelling sand fly, often abundant in the canopy at the end and beginning of the rainy seasons (91). This species normally rests on tree trunks and will attack man during the day and at night (76). Its usual hosts are arboreal mammals, especially sloths (Choloepus didactylus) and anteaters (Tamandua tetradactyla) which serve as reservoirs of this disease. Opossums (Didelphis marsupialis) have also been found naturally infected at Manaus, Brazil (6, 8) but they may represent accidental, "dead end hosts" (73).

Infection rates as high as 15.9% have been recorded in wild-caught L. umbratilis in French Guiana and there, at certain places and times of the year, it has been calculated that a person will be bitten by at least one infected L. umbratilis per hour (91). Susceptible persons entering endemic forests are at high risk; presently in Manaus, from 20 to 30 new cases of leishmaniasis due to this parasite are seen daily in clinics (J. Arias, personal communication). Secondary vectors, also in the sub-genus Nyssomyia, include L. whitmani and L. anduzei (5, 84).

Lutzomyia umbratilis also occurs in the Amazon basin of Columbia (190), Surinam (179, J. Hudson & D. Young, unpublished data), Venezuela in Bolivar State (D. Feliciangel, personal communication) and Peru (101). Human infections of Le. b. guyanensis have been reported in Surinam (179) and more recently in SE Colombia (R. Kreutzer, personal communication).

LEISHMANIA BRAZILIENSIS PANAMENSIS   Considerable information on the epidemiology of leishmaniasis caused by this parasite has been gathered in Panama during the past 35 years.   Christensen et al (24) reviewed this subject, giving references to the many detailed studies on the distribution, ecology and disease relationships of Panamanian sand flies.

The epidemiologies of leishmaniasis due to this subspecies and Le. b. guyanensis are remarkably similar (73).   Once again, arboreal mammals, especially sloths which have cryptic infections of Le. b. panamensis (52, 54), serve as principal reservoirs and Lutzomyia (Nyssomyia) spp. are the main vectors, notably L. trapidoi and L. ylephiletor.   These species have been found naturally infected in Panama, as have L. gomezi and L. panamensis (24) but their role in transmission is less clear and their geographic ranges are wider than those of the two Nyssomyia species (106) but, like them, females are highly anthropophilic.

Leishmania braziliensis panamensis also occurs in Honduras (197), Costa Rica (194, 198), Colombia (118; R. Kreutzer, personal communication) and probably Ecuador where Hashiguchi et al (47) recently isolated Le. braziliensis complex parasites from females of L. trapidoi and L. hartmanni.   The latter species was previously unsuspected as a vector of any of the leishmaniases.   Elsewhere, natural infections believed, or proven, to be Le. b. panamensis have been reported in L. ylephiletor in

Costa Rica (194) and L. trapidoi in Colombia (A. Morales, personal communication).

In Central America, from Panama to Guatemala, the previously unsuspected occurrence of L. edentula, an anthropophilic sibling species of these Nyssomyia spp., must be considered when studying vectors in this region (D. Young & C. Porter, unpublished data). In fact, one of the male paratypes of "L. ylephiletor" from Chiriqui Prov., Panama represents not that species, but L. edentula. The female characteristically has nine or more spermathecal annuli and very wide sperm ducts unlike those of L. trapidoi or L. ylephiletor. It remains to be seen whether L. edentula or the undescribed verrucarum group species in Costa Rica, mentioned earlier, are involved in the transmission of Le. b. panamensis or other Leishmania.

LEISHMANIA BRAZILIENSIS PERUVIANA   Lainson et al (79) reviewed information on the epidemiology of Le. b. peruviana in Peru and demonstrated for the first time that it is a typical peripylarian Leishmania based on its growth pattern in experimentally-infected L. longipalpis. This parasite occurs in the western slopes and in valleys of the Peruvian Andes where fewer than six phlebotomine species have been reported (56, 193). In Argentina, the presence of Le. b. peruensis has not been confirmed; no recent isolates from there have been identified as Le. b. peruensis and the sand fly fauna of that country is poorly known (106).

In Peru, where leishmaniasis ("uta") has been linked with sand flies for centuries (53), there are still many unanswered questions remaining about natural transmission cycles. Herrer (51) innoculated 97 triturated L. peruensis females into seven hamsters, two of which subsequently became

infected with leishmaniasis (no other natural infections of sand flies have been reported in Peru). The altitudinal distribution of Le. b. peruviana in the western Andes, from approximately 900 M to 3000 M above sea level (79), does not completely coincide with that of L. peruensis which is rare or absent below 1400 M (56). Lutzomyia verrucarum, on the other hand, is also a suspected vector because of its wide altitudinal range (56), abundance, peridomestic habits and aggressive man-biting behavior (56). Furthermore, another sand fly, L. oligodonta which may also be anthropophilic, was recently discovered near Cocachacra, Peru, a leishmaniasis endemic site in the Rimac Valley (123). It is possible of course, that more than one vector species transmits Le. b. peruviana in these areas.

Although domestic dogs have been found naturally infected, and sympathic foxes have been experimentally infected with this parasite (48), Lainson (73) suggested that further attempts to find Leishmania in other mammals, especially rodents, are indicated. Herrer (50) reported that 21 out of 47 sentinel hamsters, placed in caves in the Santa Eulalia Valley of Peru and exposed to sand flies, developed leishmanial lesions following eight weeks exposure. Moreover, near Trujillo, Peru, Leishmania was recently isolated from rats, Rattus rattus (L. Cruzado, personal communication) but these parasites may not be consubspecific with Le. b. peruviana based on their rapid growth in hamsters. If rodents are subsequently incriminated as reservoirs, then the role of L. noguchi as a vector among them, but not to man, must be considered.

LEISHMANIA SPECIES FROM ARMADILLOS Lainson et al (84) isolated Leishmania from blood, liver and spleen of three of 14 armadillos (Dasypus novemcinctus)

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collected in north Para State, Brazil. No skin lesions were observed. Among other observations, the hindgut, as well as, midgut development of these stocks in experimentally-infected L. longipalpis, showed that they belonged in the Le. braziliensis complex (84). Further characterization by enzyme electrophoresis demonstrated that the armadillo Leishmania differed from other members of this complex.

From wild caught females of L. ayrozai and L. paraensis in Rondonia State, Brazil, Arias et al. (7) isolated and identified seven strains of this parasite by enzyme electrophoresis. These anthropophilic sand flies, belonging in the subgenus Psychodopygus, are widespread in the Amazon basin but they have not been implicated in leishmaniasis transmission to humans.

Section Suprapylaria (Leishmania Donovani Complex)

LEISHMANIA DONOVANI CHAGASI Presently there is unresolved controversy regarding the identity of this parasite, the etiologic agent of American visceral leishmaniasis. Some authors suspect that some strains, at least, represent introduced Le. d. infantum from the Old World (68) because their enzyme profiles are essentially indistinguishable (78, 151); dogs serve as domestic reservoirs, and the incidence of infection is highest in children (178). In contrast, Jackson et al (57, 73) distinguished Le. d. infantum from Le. d. chagasi by radiorespirometry and DNA analysis. Furthermore it is argued that the widespread geographic distribution of Le. d. chagasi from Mexico to Argentina (171) indicates that it is a precinctive parasite in the Americas. An exception may be the Leishmania parasite causing canine leishmaniasis in Oklahoma and Kansas, USA (4, 107).

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The role of sylvatic mammals as additional reservoir hosts has not been fully determined but, when better understood, may help settle this controversy. Foxes (Cerdocyon & Lycalopex) in Brazil (76) and the black rat (Rattus rattus) in Honduras (73) have been found naturally infected with Leishmania, probably Le. d. chagasi.

With few exceptions (26, 107), visceral leishmaniasis due to this parasite in the Western Hemisphere is associated with L. longipalpis, the habits and disease relationships of which were reviewed by Lainson & Shaw (76). This species, a proven vector as noted earlier, occurs in semi-arid, often treeless areas from Mexico to Argentina (106). No infections of Le. d. chagasi have yet been found in Costa Rica (196) or in Panama (24). Males of L. longipalpis from Ceara State, Brazil and elsewhere (175) have two pale spots on abdominal tergites 3 & 4; whereas, those from most other localities have but one such spot on tergite 4. These probably represent the surface structure of abdominal glands that emit pheromones (85). Because of this difference and because cross-mating experiments (175) indicated that the two forms were reproductively isolated, it appears that in Brazil, at least, L. longipalpis represents a species complex. One of us (D. Young, unpublished data) examined males of L. longipalpis from Mexico, El Salvador, Costa Rica, Panama, Colombia, Bolivia, Venezuela, and Paraguay, noting that only those from the latter country (N=23 from Asuncion) represented the two spot form, which according to Ward et al (175) may be more domestic and anthropophilic than its counterpart. In any event, it is clear that females of the one spot form transmit Le. d. chagasi in many foci.

Lutzomyia longipalpis, however, does not occur at Edmond, Oklahoma or Stanley, Kansas, USA where autochthonous leishmaniasis in foxhounds have been reported (4, 107 & J. Fox, personal communication). Because *Leishmania* isolated from infected dogs has been identified as *Le. d. infantum* (28) and as *L. mexicana* (70), it is not yet clear whether one or more leishmanial taxa are involved. The disease in dogs generally is consistent with that of visceral leishmaniasis (4) but some differences have been noted (107). Additional information, provided below, was kindly provided by J. Fox (personal communication) and McKenzie (107).

Since 1979, when the first four dogs from Oklahoma were diagnosed as having leishmaniasis (4), at least 10 others have been found infected. One of these was shipped to the Edmond kennel from Stanley, Kansas. Leishmanial amastigotes were observed in this dog within two weeks after its arrival in Oklahoma; indicating therefore, that it contracted the disease in Kansas. The other infected dogs were born and raised at the kennel in Edmond. Continuous removal of infected dogs has not stopped transmission of this disease.

So far, only *L. vexator*, a reptile feeding sand fly, has been reported at the Edmond site (192) but no adequate entomological survey has been carried out there or in Kansas where no *lutzomyia* species have been reported. The apparent lack of a suitable sand fly vector lead McKenzie to study the possibility that the brown dog tick, *Rhipicephalus sanguineus*, could serve as a biological vector.

Accordingly, she allowed 500 lab-bred nymphs of this species to fully engorge on each of two naturally infected dogs - the one that had contracted the infection in Kansas; the other from the Edmond kennel.

Before and after molting to the adult stage about 30 days later, some of the ticks were examined periodically for leishmanial infections by inoculating triturated tick guts into suitable culture medium. Positive cultures from ticks that had fed on the Kansas-infected dog 160 days earlier were recorded, thus demonstrating transstadial transmission for the first time. McKenzie, however, did not microscopically observe Leishmania in serial sections of tick guts nor did she determine if they multiplied in ticks following their infecting blood meals.

Fifty other potentially infected ticks, not sacrificed for culture attempts, were allowed to refeed as newly emerged adults on two noninfected dogs. Following corticosteroid treatment one year after these ticks had fed, cultures of bone marrow from the dog exposed to ticks infected with the Oklahoma parasite were Leishmania positive. At the same time, xenodiagnosis using lab-bred ticks also demonstrated leishmanial infection. Surprisingly, other attempts to isolate or observe leishmaniae in this dog were negative. Leishmania was not detected in the other dog used in this tick uninterrupted feeding trial nor in sylvatic mammals which occur near the kennels at Edmond and Stanley (J. Fox, personal communication). Serological evidence of leishmaniasis in coyotes (Canis latrans) in nearby Texas (42) may or may not have a bearing on transmission in Oklahoma or Kansas; it must first be demonstrated that these animals actually harbor Leishmania. Moreover, the role of sand flies as vectors can not be discounted, especially in view of the occurrence of L. diabolica, a mammal feeder, in northern Texas (102).

In 1984, we had the opportunity to study the development of Le. d. infantum in experimentally-infected females of this sand fly (unpublished

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data). We allowed 57 lab-reared L. diabolica to feed on the ears, back
and stomach of a dog that had previously lived in Sicily for three years
but which had returned to Florida, USA with its owners. It was assumed
to be infected with Le. d. infantum because of its history and clinical
symptoms. Also, the dog had not lived in endemic areas, other than
Sicily. There were abundant amastigotes in the healthy-appearing skin
and in the spleen and bone marrow.

Following the infecting blood meals, the sand flies were maintained
at 27°C; 85-95% RH. An overall infection rate of 89.5% (51 out of 57
flies) was subsequently recorded. We observed massive infections of the
stomadeal valve, beginning at day 5 post feed, but not in the cibarium or
mouth parts at any time, up to 11 days after the infecting meal. Eleven
of these flies, with infections ranging from six to 11 days, refed on non-
infected hamsters and, in one case, on a BALB/c mouse, but transmission
was not demonstrated when these animals were examined 11-12 months later.
Two other hamsters, inoculated with amastigotes from bone marrow aspirates
from the same dog, were found heavily infected when they died six to
eight months after inoculation.

During the time observed (up to 11 days), the growth of this parasite
in L. diabolica was essentially identical to that observed in Phlebotomus
ariasi, a natural vector of Le. d. infantum in France (138), and in L.
longipalpis, experimentally-infected with the same parasite (68).
According to these studies, promastigotes do not ordinarily invade the
pharnyx until 10 days of extrinsic growth and they do not appear in the
mouth parts until after day 15. None of the L. diabolica in our study
survived longer than 11 days so it was impossible to determine if further

development would have occurred. However, since there was no indication of abnormal growth or parasite mortality in any of the infected flies, we regard L. diabolica as a potential vector of this disease.

Section Suprapylaria (Leishmania Hertigi Complex)

The two named subspecies in this complex, Le. hertgi hertigi and Le. h. deanei, asymptomatically infect tree porcupines in the neotropics but have not been isolated from human patients or phlebotomine sand flies (72). The nominate subspecies was described from Panamanian porcupines, Coendou rothschildi, in which infection rates as high as 86% have been recorded (49). Leishmania h. deanei parasitizes two other species of Coendou in Brazil (76).

Section Suprapylaria (Leishmania Mexicana Complex)

To date, the only proven vectors of the Le. mexicana parasites belong in the flaviscutellata complex of Lutzomyia (34, 95) but it is only a matter of time until sand flies in other species groups will be incriminated in some foci. None of the flaviscutellata complex spp., for example, occurs in south central Texas or in the Dominican Republic where autochthonous human cases of leishmaniasis caused by Le. mexicana or Le. mexicana-like parasites have been reported.

This group of Leishmania is represented by one or more taxa in many parts of the lowland neotropics and in the southern Nearctic Region of northern Mexico and Texas.

LEISHMANIA MEXICANA MEXICANA   From intensive studies carried out in southern Mexico (10) and Belize (182), mostly before 1970, our knowledge of the epidemiology of leishmaniasis caused by this subspecies is relatively well understood. It is an enzootic disease, normally circulating

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among forest rodents, especially Ototylomys phyllotis, Heteromys desmarestianus and Nyctomys sumichrasti (76). The incriminated vector is L. o. olmeca, distributed in lowland forests from Mexico to Honduras (34) but populations likely extend south into extreme western Panama (J. Murillo & D. Young, unpublished observations). In the neotropics, however, the disease is known only in Mexico, Belize, Guatemala and possibly Honduras (171). The risk of human infection is especially great during the rainy seasons when adult populations of L. o. olmeca are high (182). Disney (73) found the first naturally infected female of this subspecies in Belize; others were subsequently discovered in that country (182) and in Mexico (10). The habits of this sand fly were reviewed by William (182) and Lawson & Shaw (76).

Recently, a wild-caught female of L. ylephiletor was found infected with Le. m. mexicana in Izabal Dept., Guatemala (C. Porter, personal communication). The identification of the strain was confirmed by enzyme electrophoresis (R. Kreutzer, personal communication). The significance of this finding in a highly anthropophilic sand fly, unlike L. o. olmeca, can only be determined when further studies are undertaken.

Leishmania m. mexicana or a very similar parasite (70) also occurs in northern Mexico and south central Texas, USA where the climate and vegetation differ sharply from those of neotropical foci. There is now little doubt that the human cases of cutaneous and diffuse cutaneous leishmaniasis reported in Coahuila State, Mexico (3 cases; 128) and in Texas (8 cases; 44) were autochthonous. In 1985, a boy from Uvalde, Texas and a domestic cat from the same city, but from different households, were parasitologically confirmed as having cutaneous leishmaniasis

(F. Gustafson, personal communication). Leishmaniae isolated from them are now being biochemically identified. Neither the boy nor the cat had previously travelled to leishmaniasis-endemic areas outside of Texas.

Lutzomyia diabolica, closely related to, but distinct from, L. cruciata (192), is the suspected vector in these foci because it is the only known anthropophic sand fly present (;92); its geographic distribution parallels that of known human infections (44); and females are able to transmit the parasite following extrinsic development under experimental conditions (86). The latter evidence for vector incrimination is given less weight than the others because L. anthophora and L. shannoni females are also able to experimentally transmit one of the Texas-isolated parasites (86, 192). The former species is not anthropophilic (32) and L. shannoni does not exist in these endemic areas (192). To date no natural leishmanial infections have been found in Texas sand flies or in vertebrates other than man and the domestic cat.

It is significant that most patients in Texas probably contracted the disease in late summer or during autumn (44) when the proportion of parous, and thus infective, flies may be the highest (43). In Uvalde County, Texas, adults of L. diabolica has been found from 17 May to 14 December (86). Other information on the habits of this species (86) include the following. Females are agressive man-biters, attacking man mostly during the evening hours but occasionally during the day as well. The species is exophilic and endophilic and is commonly found resting in houses and outbuildings. The natural resting sites and host preference range remain unknown. Females are anautogeneous and gonotrophically concordant. Nearly all eggs laid by late season females (November to December) will not hatch

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until 90 to 270 days later. In contrast, those laid by females in June and July all hatch within five to 25 days following oviposition. Specimens may also overwinter as mature larvae.

Of 523 lab-bred L. diabolica females that experimentally fed on leishmaniasis-infected hamsters (Texas strain, WR-411), 460 (87.9%) became infected (86). Suprapylarium development was confirmed in all females that died before refeeding. Short, active promastigotes were observed in the mouth parts beginning as early as day 4 post feeding. Twenty-five females survived the first oviposition to refeed on 21 non-infected hamsters, five of which subsequently developed leishmanial lesions from 25 to 148 days after being bitten. It was shown that flies, previously fed on infected hamsters from four to seven days earlier, were able to transmit this strain of Leishmania by bite to naive hamsters (86). LEISHMANIA MEXICANA AMAZONENSIS From the extensive studies undertaken in Para State, Brazil (76, 132, 155, 173, 174), much information has been obtained on the natural transmission cycle and vector relationships of Le. m. amazonensis, considered by some to be identical to Le. m. pifanoi (116). This parasite is transmitted to terrestrial rodents, especially Proechimys guyannensis and to a lesser degree to other mammals by L. flaviscutellata, a sand fly only slightly anthropophilic (76). For this reason, human disease due to this Leishmania is relatively rare, accounting for fewer than 3% of all leishmaniasis cases seen at one laboratory in Belem, Brazil from 1965 to 1982 (73). Both simple cutaneous, and a suprisingly high proportion (ca. 30%), of diffuse cutaneous leishmaniasis are caused by Le. m. amazonensis (73).

Up to 1982, 45 out of 7,498 _L. flaviscutellata_ females examined had natural infections of this _Leishmania_ (73). Differences in infection rates of these sand flies collected within 300 m apart in North Brazil suggested that "hot spots" of infections exist, probably because of the proximity of infected resorvoir hosts (76). During these investigations, laboratory colonies of _L. flaviscutellata_ were established (170) and experimentally-infected females transmitted _Le. m. amazonensis_ by bite to hamsters (174).

Elsewhere, _Le. m. amazonensis_ has been isolated from spiny rats, _Proechimys_, in French Guiana (J.P. Dedet, personal communication) and probably from a _L. flaviscutellata_ female in southern Venezuela (124). Yet, the identity of the sand fly from Venezuela was never confirmed and the possibility exists that it may have represented one of the _L. olmeca_ taxa, an undescribed subspecies of which was recently discovered in Amazonas, Venezuela (D. Feliciangeli, personal communication).

This _Leishmania_, or a similar one, was definitely found in field-collected females of _L. flaviscutellata_ in Trinidad (165) but no natural infections in this species have been reported in Colombia, Surinam, French Guiana, Ecuador and Peru, near the Bolivian border, where _L. flaviscutellata_ is known to occur (106, 192). Recent characterization of _Le. m. amazonensis_ from human patients in Colombia (R. Kreutzer, personal communications) indicates, not surprisingly, that this parasite may be widely distributed in the Amazon basin.

LEISHMANIA MEXICANA ARISTEDESI At present, this distinctive subspecies (24) is known only from Sasardi, San Blas Province, Panama, where Herrer et al (55) identified infections in wild-caught rodents, especially

Oryzomys capito (14 positive out of 39 examined). Nearly all sand flies (99%) captured in rodent-baited Disney traps at this lowland site (22, 159) represented L. o. bicolor, a subspecies also known from Costa Rica, Colombia, Venezuela and Ecuader (12, 190). This sand fly may also occur in some parts of Brazil, Peru and possibly Bolivia but more collections are needed for confirmation (D. Young, unpublished data). Christensen et al (22) failed to find natural infections of Le. m. aristedesi in 449 female L. o. bicolor dissected and in over 1000 females pooled, but, nevertheless, they considered that this sand fly is the probable vector at Sasardi due to its feeding habits, abundance and close taxonomic relationship to proven vectors in the L. flaviscutellata complex. It is not a common man-biter in Panama (24) and Le. m. aristedesi has not yet been isolated from humans there or elsewhere.

LEISHMANIA MEXICANA ENREITTII FROM BRAZIL   This subspecies parasitizes domestic guinea pigs and Lutzomyia monticola has been experimentally infected (102); both the sand fly and the parasite occur at or near Curitiba, Panama State, Brazil (73), but further studies are needed to incriminate this sand fly as a natural vector.

LEISHMANIA MEXICANA GARNHAMI   The taxonomic status of this parasite, isolated from human patients and from a Didelphis opossum (73, 153) in Trujillo State, Venezuela, remains questionable. It has been determined that strains from these are indistinguishable from Le. M. amazonensis by enzyme electrophoresis and other analyses (116). Moreover, the possibility exists that a Le. brasiliensis subspecies may also occur in the endemic zone owing to the slow in vitro growth of some stocks and because of observed peripylarian growth in some experimentally infected sand flies (119).

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However, it is clear that the suspected vector is an undescribed _Lutzomyia_ sp. in the _verrucarim_ species group and not _L. townsendi_ as previously believed (D. Feliciangeli, J. Murillo & D. Young, unpublished data). This sibling species of _L. townsendi_ also occurs in Costa Rica where it is also suspected of being a leishmaniasis vector (R. Zeledon, personal communication).

The altitudinal range of this anthropophilic species in the Andes of western Venezuela correlates well with that of human infections, mainly from 800 M to 1800 M above sea level where it is the most commonly encountered sand fly (114). Further information on its habits and relationship to _Leishmania_ is given in other articles (17, 105, 119, 152). At present, no naturally infected females have been reported from western Venezuela.

Finally, we add that presently there is no basis for the suggestion (73) that a species in the _L. flaviscutellata_ complex may be involved in the transmission of _Le. m. garnhami_, or other _Leishmania_, at these relatively high elevations.

LEISHMANIA MEXICANA PIFANOI   There is mounting evidence that this parasite may be consubspecific with _Le. m. amazonensis_, and, if confirmed, then the latter taxon will become a junior synonym (116). _Leishmania m. pifanoi_ has been isolated only from human patients suffering from diffuse cutaneous leishmaniasis in Venezuela (72).

LEISHMANIA MEXICANA VENEZUELENSIS   Since 1974, 73 human cases of leishmaniasis caused by this parasite have been reported in the Barquisimeto, Venezuela area (12). At one site, "Bosque de Macuto," six anthropophilic _Lutzomyia_ spp., including _L. olmeca bicolor_, have been

captured (12). There is no other information on potential vectors.

LEISHMANIA MEXICANA SUBSPECIES FROM MATO GROSSO STATE, BRAZIL   This
apparently distinct Leishmania (72) is known only from strains isolated
from human patients (72) amd there is no information on vectors.

LEISHMANIA MEXICANA SUBSPECIES FROM MINAS GERAIS STATE, BRAZIL   This
parasite has been isolated from man and dogs and, again, nothing is
presently known about associated vectors (72).

LEISHMANIA SPECIES FROM THE DOMINICAN REPUBLIC   Bogaert-Diaz et al. (11)
first reported autochthonous human cases of leishmaniasis from the
Dominican Republic in 1975.   Each of three brothers had diffuse cutaneous
leishmaniasis (DCL), a relatively rare condition observed in individuals
with deficient cell-mediated immunity and, in the New World, associated
with L. mexicana parasites (178).   Lainson (73) reported that one
leishmanial strain from the Dominican Republic showed typical suprapylarian
development in experimentally-infected L. longipalpis.   Further character-
ization indicated that the Dominican Leishmania is closely related to
other Le. mexicana parasites but, nevertheless, differs in enzyme profiles
and other characteristics (150).

Since 1975, 22 additional patients, all presenting DCL, have been
parasitologically confirmed in the Dominican Republic (61).   At least 16
of them probably, or certainly, contracted the disease in the eastern
mountains (Cordillera Oriental) in the provinces of El Seibo and Altagracia
(61).   No simple ulcerated lesions have yet been observed.

In 1949, Fairchild & Trapido (35) collected two Lutzomyia spp. in
Hispaniola which they described and named L. christophei (verrucarum
species group) and L. cayennensis hispaniolae (cayennensis species group).

They remain the only extant sand flies known from Hispaniola.

In view of this unique and little-known focus of leishmaniasis, Johnson (61) conducted a sand fly survey in the Dominican Republic at various times from 1981 to 1983. The following comments are based on his observations except where otherwise cited.

Of 17 case sites visited one or more times, Johnson noted that all were associated with shady coffee/cacoa groves which, in effect, simulate forests which once covered most of the island. Residents at some of these case sites are aware of biting flies ("erisos"), describing them as small and pale with a hopping flight that begin to bite at dusk, inside and outside of houses. The good possibility that "erisos" were, indeed, phlebotomines was confirmed when a DCL patient collected two female "erisos" biting him in September, 1983. The flies were later identified as L. christophei by R. Johnson and D. Young. To date, this observation remains the only one showing that L. christophei females bite people under natural conditions.

Other observations from Johnson's study support the hypothesis that this species is the probable vector. In contrast to L. c. hispaniolae which feeds on reptiles (mostly lizards), females of L. christophei are mammalophilic and were observed to feed on wild caught rats, Rattus rattus, albino laboratory mice and hamsters. Lab-reared females probed the skin of human volunteers but did not take full blood meals.

Furthermore, one leishmanial stock (Isabel strain, WR 336) developed and multiplied well in experimentally-infected females of L. christophei, showing typical sypropylarian growth. Eight of these 52 females, fed previously on infected BALB/c mice, took second blood

meals from three noninfected mice seven to 12 days after the infecting feeds. Four weeks later, one of the mice had a slight swelling of the hind foot where one of the L. christophei females had earlier fed. Xenodiagnosis using lab-bred females of L. anthophora demonstrated leishmanial infection. In addition, in vitro cultures of hind foot aspirates from this mouse and from spleen tissue of the two others were also Leishmania positive.

Johnson collected small numbers of L. christophei at or near seven human case sites from tree holes, on tree trunks and in rock crevices. At no time during the study were they common; 23 wild-caught females were examined for leishmanial infections but none was positive. No flies were captured in hamster-baited Disney traps, placed close to known resting sites nor were any collected from human bait. However, these collection techniques were not systematically used during the entire study period.

The natural hosts of L. christophei remain unstudied. Introduced murine rodents, Rattus and Mus, have largely replaced native rodents which may have served as the original hosts of this Leishmania. The only two remaining mammals that are precinctive to Hispaniola are Plagiodontia aedium, a large secretive rodent, and Solenodon paradoxus, an insectivore (187). Both species are uncommon and do not live in close proximity to man. Other potential reservoirs include the introduced mongoose, dogs, feral cats or, less likely, man himself. Johnson using the indirect fluorescent antibody test, found that four out of 44 Rattus rattus, captured near human case sites, were seropositive for leishmanial antibodies but no amastigotes were observed or cultured from any of them.

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CONCLUDING REMARKS

In general, nearly all topics covered or not covered in this chapter require considerable more attention. It is apparent that long term epidemiological studies are needed for a complete understanding of leishmanial transmission cycles and to monitor changes in disease incidence brought about by changes in habitat. Such commitments are essential for the formulation of control measures which, with few exceptions, have been completely unsuccessful in the New World (104).

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Table 1. Suspected or proven vectors of leishmaniasis in the New World[a]

Lutzomyia subgenus or species group	Lutzomyia species or subspecies	Associated Leishmania	Where suspected or incriminated as a vector	Reference
Subgenus Lutzomyia	diabolica	Le. mexicana	Mexico & USA (Texas)	86
	gomezi	Le. b. panamensis	Panama	24
		Le. b. braziliensis	Colombia	present chapter
	longipalpis	Le. d. chagasi	Mexico to Argentina	83, 90
migonei species group	migonei	Leishmania	Brazil, Venezuela	76
	anduzei	Le. b. guyanensis	Brazil, Guyanas	5, 76
	flaviscutellata	Le. m. amazonensis	Brazil, Venezuela, Trinidad	73, 124, 165
Subgenus Nyssomyia	intermedia	Leishmania	Brazil	38
	olmeca olmeca	Le. m. mexicana	Mexico, Belize, Guatemala	10, 182
	olmeca bicolor	Le. b. aristedesi	Panama	22
	trapidoi	Le. b. panamensis	Panama, Colombia,	24, 118
		Leishmania	Ecuador	47
	umbratilis	Le. b. guyanensis	Brazil, Guyanas	76, 121
	whitmani	le. b. guyanensis	Brazil, Guyanas	5, 76
		Leishmania	Brazil	76

Table 1. (Continued)

	ylephiletor	Le. b. panamensis	Panama, Costa Rica	24, 194
		Le. m. mexicana	Guatemala	C. Porter, personal communication
Subgenus Pintomyia	pessoai	Leishmania	Brazil	38
Subgenus Psychodopygus	ayrozai	Leishmania of armadillos	Brazil	7
	panamensis	Le. b. panamensis	Panama	24
		Leishmania	Venezuela	125
	paraensis	Leishmania of armadillos	Brazil	7
	wellcomei	Le. b. braziliensis	Brazil	73, 81
verrucarum species group	christophei	Leishmania	Dominican Republic	61
	spinicrassa	Le. b. braziliensis	Colombia	present chapter
	verrucarum	Le. b. peruviana	Peru	79
	unnamed species	Le. m. garnhami	Venezuela	153
vexator species group	hartmanni	Leishmania	Ecuador	47
	peruensis	Le. b. peruviana	Peru	51

[a]See text and other references (7, 47, 76, 144) for more details on these and other Lutzomyia species, some of which have been found naturally infected with unidentified promastigotes. Parasites, listed here as Leishmania only, are either unnamed or have not been specifically identified. Not all of the leishmanias listed above have been isolated from sand flies.

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